Bluetongue virus outbreak in Tamil Nadu, southern India: Need to study the Indian biting midge vectors, *Culicoides* Latreille (Diptera: Ceratopogonidae)

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Bluetongue (BT) is a viral disease causing morbidity and mortality in sheep, cattle and wild ruminants, including deer, sambar and bluebull. BT is endemic in many parts of India and its outbreak in Tamil Nadu during the monsoon season of 1997–98 caused the death of 300,000 sheep and goats. However, the extent of damage to the wild ruminants remains unknown. Current research for controlling BT is confined to the development of vaccines in disease vulnerable states, with which complete success is questionable because of the presence of more than 20 serotypes of the virus. Vector control through integrated, intensive management is feasible, but there is little knowledge of the biting midges, Culicoides Latreille (Diptera: Ceratopogonidae) in southern India. Culicoides brevitasis Keiffer and C. imicola Keiffer are proven vectors of BT virus, occurring widely in India and other parts of the Oriental region. There is an urgent need to study Culicoides sp. intraspecies variation and genetic variation because of the recent BT disease outbreak. This information will provide an understanding about BT transmission and control and vector competence of Culicoides in India. This article presents the first report of BT in Tamil Nadu over the past 12 years (1988–99), reviews the current knowledge of Indian Culicoides fauna, and how the lack of understanding of taxonomy, biology and behaviour of this fauna affects effective vector control measures.

Keywords: Bluetongue virus, biology, control, *Culicoides* sp., taxonomy.

CULICOIDES sp. (Diptera: Ceratopogonidae) are commonly known as biting/bloodsucking midges or gnats. These midges are of great concern because they transmit bluetongue (BT), Akabane and other viruses that cause disease in sheep, cattle and wild ruminants[1,2]. The recent outbreak of BT disease in Tamil Nadu and its occurrence in many parts of India over the last few decades has affected millions of sheep and goats and other livestock. India earns Rs 8500 crores annually through the production of meat, wool and skin from 485 million livestock, of which Tamil Nadu accounts for 24 million livestock (Seventeenth Livestock Census 2003, http://dahd.nic.in/census/liv97.xls). Small and medium farmers in Tamil Nadu rear sheep and goats for meat and skin, which fetches Rs 200 for an individual animal, and the current total number of sheep and goats records 5.2 million and 6.4 respectively. Sustained growth of the sheep and goat industry depends on the absence of diseases such as BT. However, knowledge of *Culicoides* sp. and its vector species in relation to transmission of BT is poorly known in India. This article presents the occurrence of BT in Tamil Nadu over the past 12 years (1988–99), reviews the current knowledge of Indian *Culicoides* fauna, and suggests the urgent need to study taxonomy, biology and behaviour of Indian vector species in order to adopt effective vector control measures.

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Outbreak of BT disease in Tamil Nadu

BT disease continues to be endemic to cattle and wild ruminants in Africa, where the greatest number of host animals, vectors and virus strains were identified. It was first recognized in cattle and sheep in South Africa in the early 1930s and has been found in temperate to sub-tropical areas worldwide. It was reported from Cyprus in 1943, Israel in 1951, USA in 1954, Portugal in 1956, Spain in 1957, and Australia in 1974. The extent of BT infection in domestic and wild ruminants in South and South East Asia is unknown, but its actual and potential occurrence there is a matter of serious concern[3].

BT in India is endemic to Tamil Nadu, Andhra Pradesh, Karnataka, Maharashtra, Gujarat, Rajasthan, Haryana, Himachal Pradesh and Jammu and Kashmir. In Tamil Nadu (Figure 1), 22 out of 23 districts were reported to be affected by the BTV (blutongue virus). Dr Leelavathy, former Director of Veterinary Services, Tamil Nadu, readily provided unpublished, raw data on the annual incidence of BTV from Tamil Nadu. I have reanalysed the data consecutively from 1987–88 to 1998–99, and the results are presented in Figures 2 and 3. The reported cases of BTV among sheep and goats occur presumably in an epidemic form during the monsoon season. Although the history of reporting was not continuous, the number of outbreaks, attacks and deaths among ruminants reported is of great concern, that needs immediate attention for the protection of livestock and economic growth. The pattern of the disease in this state can be divided into three parts: (i) the northeastern part that includes only Chennai (=Madras) district, the state headquarters where no cases of BTV were reported; (ii) the northern and central districts and Kanyakumari district in the south with incidence of BT from a few cases to less than one thousand, and (iii) the southern districts that recorded annually more than one thousand cases, hence they are 'hyperendemic' for BT. During 1997–98

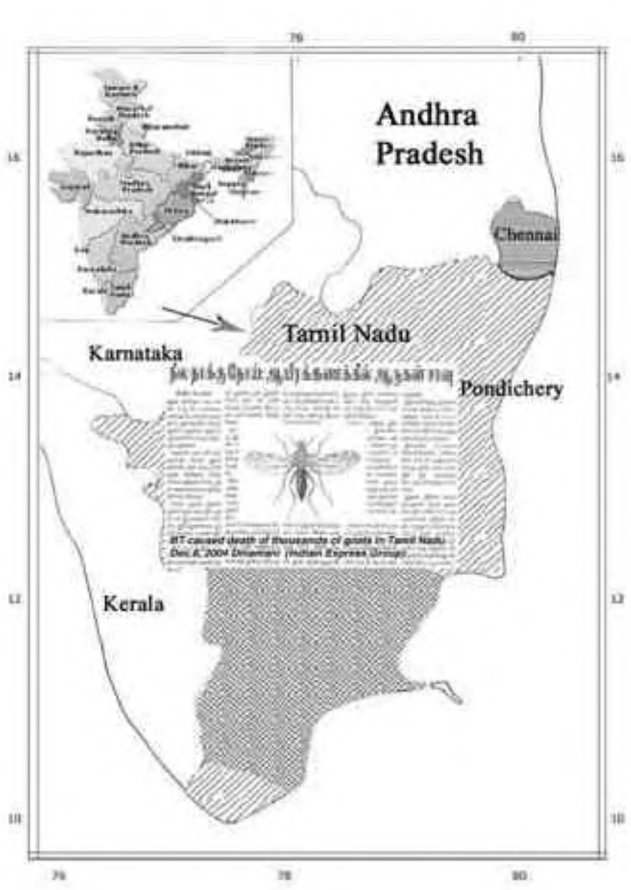

Figure 1. Endemic areas of bluetongue in sheep and goats with no cases (horizontal slashes), < 1000 cases (oblique slashes), > 1000 cases (crossed slashes) recorded from Tamil Nadu from 1987–88 to 1998–99.

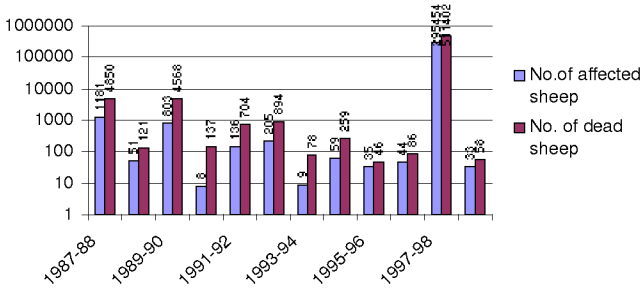

Figure 2. Incidence of bluetongue in sheep and goats from 22 districts of Tamil Nadu from 1987–88 to 1998–99 (actual number of cases plotted on bar diagrams drawn in log value).

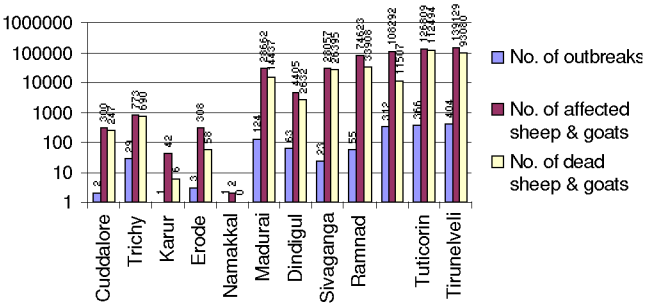

Figure 3. Outbreak of bluetongue in 12 districts of Tamil Nadu during the monsoon season of 1987–88 with the number of affected and dead sheep and goats (actual number of cases plotted on bar diagrams drawn in log value).

from 12 districts in southern Tamil Nadu, outbreak of BT in sheep and goats swept in an epizootic form leaving alarmingly 523,203 infected and 298,018 dead (Figure 3). The extent of infection and disease in wild ruminants was completely unknown. Details of livestock losses due to BT infection during this time from other states are unknown, although Prasad *et al.*[4] have reported BT in livestock from northern India, where transmission of BT is still active.

BT research in India is focused on vaccine development against the disease. Institutions involved from different parts of the country include: Centre of Animal Health Studies, Tamil Nadu University for Veterinary and Animal Sciences, Chennai; Veterinary College at Tirupati, Acharya N. G. Ranga Agricultural University at Andhra Pradesh; Institute of Veterinary Biological in Bangalore; and BT Laboratory, C.C.S. Haryana Agriculture University, Hisar. Previous experiences revealed that the use of vaccines for controlling BTV is of limited value[5].

Entomology

Taxonomy

For any vector-borne disease control programme, a detailed study of its taxonomy and biology is essential. It is perti-

nent to quote Murray and Dyce[6] here, 'Overseas experience has shown that an essential for all epidemiological studies is a good taxonomic knowledge of the organisms under study, whether they are the vectors or the infective agents. Such studies are not fashionable at present in certain disciplines, even though resolutions are passed regularly at all international congresses on zoological topics, such as entomology, to emphasize the shortage of taxonomists and the basic necessity of such studies to those on epidemiology'. India is no different from other countries, particularly in the case of *Culicoides* fauna; there are few entomologists with the expertise to identify them and few new entomologists learning *Culicoides* taxonomy worldwide.

Culicoides sp. are small flies (1–2 mm, Figure 1 inset), black or brown-coloured having wings speckled with light and dark patterns and are proven or suspected vectors of a number of animal diseases and human pathogens[1,2]. Over 1000 species of *Culicoides* are known from around the world[7]. The number of species described from East Africa[8], South West Asia[9] and South East Asia[5] are 61, 100 and 168 respectively, while *Culicoides* fauna from South Asia remains unexplored. Of these, 27 species were recorded in India[5,10] (Table 1). However, additional taxonomic studies of Indian *Culicoides* species are needed, because even a small country like Laos has recorded 62 species[11]. The proven vectors of bluetongue are *Culicoides brevitarsis* Kieffer and *C. imicola* Kieffer in the Old World and *C. variipennis* (Coquillett) *sensu lato* in the North America[12,13]. *C. brevitarsis* is distributed widely in Australia, India to Indonesia, New Guinea, the Philippines, Taiwan, Ryuku Islands, Sri Lanka, whereas *C. imicola* occurs in Africa, the near and Middle East, India, Sri Lanka, Laos, Thailand, Vietnam and Mediterranean region. It is possible that one or both of these species of *Culicoides* could have been involved in the transmission of BT in Tamil Nadu, but to date no entomological studies are available. Prasad *et al.*[4] reported that *C. oxistoma* Kieffer is a potential vector of BT in northern India. This is unlikely as it was never recorded as a vector anywhere from the endemic regions in South and Southeastern Asia, and there were no reports of BT virus. Knowledge of intraspecies variation and genetic variation in *C. sonorensis* provided evidence for variation of vector competence (i.e. vector susceptibility to virus infection, transmission ability) and BT disease epidemiology in USA[14]. Recent taxonomic evidence based on morphological and electrophoretic studies from the Neoarctic region suggests that *C. variipennis* is a complex of three sympatric sibling species: *C. variipennis sensu stricto*, *C. occidentalis* Wirth & Jones and *C. sonorensis* Wirth & Jones[15]. Similar studies involving taxonomic status, geographical distribution and genetic variation of *C. brevitarsis* and *C. imicola* in India are needed urgently to understand vector competence, virus transmission and disease control. Such information will help veterinary and wildlife biologists to develop effective control measures during epizootic of BT in India.

Biology

Understanding of the life cycle of *Culicoides* is important. Some of the valuable contributions to our knowledge included in biology of vector species are by Kettle[1], Campbell and Kettle[16], Linley *et al.*[2] and Boorman[7].

Culicoides sp. are found in a wide variety of habitats ranging from moist compost or leaf litter to mud at the margins of ponds, lakes, streams or floating weed. Some BT vector species lay eggs in animal dung pats and manure piles; adult flies begin emerging from the dung pats 11 days after oviposition. The life cycle is completed by 24 days. Dormancy, either as diapause or aestivation, occurs in either eggs or larvae. Males form leks during dusk and females fly through to mate. Some species of *Culicoides* are known to produce sex-attractant and contact mating pheromone. Blood-meal sources are sheep, cattle, horse

Table 1. Checklist of the genus *Culicoides* in India

Subgenus *Trithecoides* Wirth and Hubert
anophelis Edwards
macfiei Causey
palpifier Sen and Das Gupta
flaviscutatus Wirth and Hubert
raripalpis Smith
Subgenus *Hoffmania* Fox
indianus Macfie
innaxious Sen and Das Gupta
peregrinus Kieffer
recurvus Delfinado
Subgenus *Avaritia* Fox
actoni Smith[a]
brevitarsis Kieffer[b,c]
dumdumi Sen and Das Gupta
fulvus Sen and Das Gupta
imicola Kieffer[b]
orientalis Macfie
Subgenus unplaced
dryadeus Wirth and Hubert
clavipalpis Mukerji
distinctus Sen and Das Gupta
huffi Causey
similis Carter, Ingram and Macfie
oxistoma Kieffer[a]
shortii Smith and Swaminath
majorinus Chu
spinulosus Chu
Subgenus *Meijerehlea* Wirth and Hubert
arakawae (Arakawa)[d]
Subgenus *Beltranmyia* Vargas
circumscriptus Kieffer
Subgenus *Pontoculicoides* Remm
kamrupi Sen and Das Gupta

[a]Vectors of cattle filaria *Oncocerca gibsoni* (Cleland and Johnston); [b]Vectors of bluetongue virus disease; [c]Vector of Akabane disease (similar to BTV); [d]Vector of fowl fox virus disease.

and wild ruminants and rarely man. Females collected after feeding on hosts were always mated. Spermatophores are formed during insemination. Autogeny, the production of eggs without first taking blood, is known in some species of *Culicoides*[2] and might play a part in population maintenance in the absence of suitable hosts for blood-meals. However, *C. brevitarsis* is not autogenous, avoiding prolonged larval food reserves, and has based its survival strategy by selecting breeding sites like dung pats in the vicinity of constant supply of hosts (sheep, cattle, etc.), which could supply repeated blood-meals. Survival rates in midges are determined by parous rates – adults that have laid eggs at least once are distinguished by the development of a dark red pigment in epidermal or subepidermal layers of the abdomen. About 150–200 eggs are laid at each oviposition. The blood-meal to oviposition (gonotrophic) cycle of *Culicoides* was studied by Linley *et al.*[2].

Dispersal rates of midges are important factors to understand the spread of infected flies and to develop effective control strategy for disease control. *Culicoides* is known to travel a short distance of 2–3 km from the breeding places. There is increasing circumstantial evidence that infected midges can be wind-borne over considerable distances. BTV and Akabane viruses might have been carried to western Turkey by midges from Cyprus and to eastern Turkey from Syria[17] and from Cuba to Florida[18].

Culicoides sp. as BT vectors

Jones *et al.*[3] illustrated a good summary of the disease transmission, and Wirth and Hubert[5] presented the role of *Culicoides* sp. in transmitting BT disease. Cattle are the primary hosts of BTV and the endemic cycle is congenital from either parents. The foetus is susceptible to virus infection and shows acute disease symptoms. If the calf survives to become an apparently healthy adult, it will remain infective for life. In the endemic cycle vector species do not cause a problem, but only in an epizootic cycle where a midge takes blood from an infected animal. After an incubation and multiplication period, infected female midges transmit virus to any wild or domestic ruminant that it will feed on, sheep, goat, cattle, deer, antelope, etc. If a high population of midges is present, the secondary or epizootic cycle may go on repeatedly from year to year. Among the ruminant hosts, morbidity and mortality are negligible in goats, in-apparent to very low in cattle, variable from moderate to high in sheep, and extremely high in deer.

The loss of sheep and other ruminants in livestock industry in the epizootic cycle was brought under control by a widespread immunization programme. But BT was still found in cattle; it had caused many abortions, still births and deformed or brain-damaged calves and thus losses in the cattle industry, especially in dairy herds. This was further complicated when the semen of highly productive and prized breeding animals were imported to be used for herd improvement at relatively low cost in underdeveloped countries. Elimination of BT from contaminated or infected sperm was found to be ineffective; hence all herds must be kept in strict quarantine. The presence of more than 20 antigenic types of BTV in a country like Kenya complicates the effectiveness of polyvalent vaccines usually containing six serotypes[19]; the use of vaccines is of limited value. Current BT vaccines are attenuated-live vaccines and could release infectious virus into cattle herds. Immunization as a form of BT control is now discouraged and thus animal breeders must turn into insect vector control to stop the epizootic cycle.

Possible causes for BTV outbreak in Tamil Nadu

Certain hypotheses regarding the source of BT resulting in BT outbreak in Tamil Nadu can be drawn by reviewing the animal breeding programme and the biting midges involved in transmitting the virus. In Tamil Nadu, especially in the southern districts, animal-breeding research centres are involved in collaboration with foreign countries for improving the quality of dairy, meat, skin, wool, tourism (rare and endangered species under breed loan programme in zoos and parks) industry. Thus a possible source of BT could be from the imported, infected semen or adult animals. A serological study might confirm this possibility. In rural areas, dung pats (used as an alternative to firewood) and manure piles (used for horticulture, agriculture nurseries and also for the production of biogas, methane gas for cooking) are the potential breeding grounds for BTV vector species. Possibly these species could breed on the dung pats and manure piles, during incessant rains of the monsoon season. Furthermore, strong winds, especially in the cyclone-hit areas of costal districts in southern Tamil Nadu could facilitate wind-blown movement of infected midges. Further studies are also required to ascertain these possibilities.

Vector control

Indian socio-economic factors and methods to ensure community participation must be considered when developing *Culicoides* control and management strategies, such as insecticide spray, weather forecasting, and habitat removal. *Culicoides* sp. are susceptible to a wide range of insecticides, including permethrin and deltamethrin that could be used effectively to control adult and larval populations. However, wind-borne reintroduction of adult flies was spread to treated areas, insecticide spray failed to achieve the desired results. Furthermore, insecticide spray is often not environment-friendly. Some of the other physical measures that can be used to control vectors of BT are the use of repellent-treated screens or netting, clothing impregnated with repellents or insecticides, and the use of

cattle (the preferred host for *C. imicola*) to protect from infection or sheep with bluetongue[7]. There are no known biological control methods for biting midges. Models developed from satellite-derived climate variables have been used for prediction of the distribution and abundance of the main European BT vector *C. imicola*[20,21]. Climate and environmental variables like temperature, soil moisture and vegetation were predicted to influence the breeding sites of BT vector and provide the sources of disease emergence and transmission dynamics of BT. Timely announcement of weather forecasting is useful for the owners of sheep and cattle. If the village community is given timely advise not to use dung pats and manure piles indiscriminately, this may result in protection of livestock from the breeding sites of biting midges during monsoon seasons and thus avoid outbreaks of BT.

Sterile insect technique (SIT) is considered to be cost-effective in some of the insect vectors, pest management and control. In Mexico, SIT was used successfully to control the screw-worm fly, *Cochliomyia hominivora* causing animal myiasis in cattle and to control the fruit fly, *Ceratitis capitata* causing a major economic loss in the fruit and vegetable industry. Now SIT is being revived to control mosquitoes, *Anopheles* sp. causing malaria[22]. This method should be considered for controlling *Culicoides* sp. to BT control.

To conclude, taxonomy of *Culicoides* sp. and its biological studies, including larval habitats, host-seeking, mating, blood-feeding, dispersal and migration, resting and oviposition are important for understanding disease transmission, longitudinal entomological intervention and disease control. Such studies are required to control the vector species and to stop the transmission of the disease during outbreak of BT.

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